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4.5. Quantification of NETs by Fluorescence Spectrophotometry

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An abbreviated version of this protocol was published in International Journal of Molecular Sciences in Jul 2021

Metabolic Pathways Involved in Formation of Spontaneous and Lipopolysaccharide-Induced Neutrophil Extracellular Traps (NETs) Differ in Obesity and Systemic Inflammation

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Detailed protocol

Quantification of NETs by Fluorescence Spectrophotometry:

Extracellular DNA was stained with 5 μM SYTOX green (Invitrogen, Carlsbad, CA, USA), a fluorescent membrane-impermeable DNA dye. Fluorescence was quantified using a microplate reader at 485/535 nm.

1) Seed the cells at the proper density (e.g. 50 000/ml) in HBSS (+) medium in 96-well plate (non-coated)
2) Stimulatate cells (e.g. with LPS)
3) At the end of the incubation period prepare SYTOX green solution (Add 1μl 5 mM stock solution to the 999 μl HBSS (+), 1:1000 ratio, 5μM = working solution) and vortex.
4) Add 10 μl of the Sytox green working solution to each well
5) Tap the plate on each side to distribute the SYTOX evenly in each well
6) Measure the plate in a microplate reader at 485/535 nm (e.g. Tecan, Infinity F200 Pro)

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Cichon, I. , Ortmann, W. and Kolaczkowska, E. (2022). 4.5. Quantification of NETs by Fluorescence Spectrophotometry. Bio-protocol Preprint. bio-protocol.org/prep1817.
2. Cichon, I., Ortmann, W. and Kolaczkowska, E.(2021). Metabolic Pathways Involved in Formation of Spontaneous and Lipopolysaccharide-Induced Neutrophil Extracellular Traps (NETs) Differ in Obesity and Systemic Inflammation. International Journal of Molecular Sciences 22(14). DOI: 10.3390/ijms22147718